

WHAT IS CLAIMED IS:

1. A recombinant construct comprising:

(a) a DNA sequence encoding a polypeptide having 3-hydroxy-3-methylglutaryl-Coenzyme A reductase enzyme activity, and

5 (b) a DNA sequence encoding at least one polypeptide having steroid pathway enzyme activity selected from the group consisting of:

squalene epoxidase enzyme activity,
sterol methyl transferase I enzyme activity,
10 sterol C4-demethylase enzyme activity,
obtusifoliol C14 α -demethylase enzyme activity,
sterol C5-desaturase enzyme activity, and
sterol methyl transferase II enzyme activity.

2. The recombinant construct of claim 1, further comprising at least one promoter operably linked to said coding regions.

3. The recombinant construct of claim 1, further comprising a first promoter operably linked to said DNA sequence encoding a polypeptide having 3-hydroxy-3-methylglutaryl-Coenzyme A reductase enzyme activity and
5 a second promoter operably linked to said DNA sequence encoding at least one polypeptide having steroid pathway enzyme activity, wherein said first and second promoters may or may not be the same.

4. The recombinant construct of claim 2 or 3 further comprising an operably linked transcription termination sequence located 3' to each coding region.

0985723 062004
100250 02/58850

5. A recombinant construct according to claim 3 wherein the promoters are selected from the group consisting of seed-specific promoters, organ specific promoters and constitutive promoters.

6. A recombinant vector comprising operably linked in the 5' to 3' direction,

a promoter, a DNA sequence encoding a polypeptide having 3-hydroxy-3-methylglutaryl-Coenzyme A reductase enzyme activity, and a transcription termination signal sequence;

a promoter, a DNA sequence encoding at least one polypeptide having steroid pathway enzyme activity selected from the group consisting of

10 squalene epoxidase enzyme activity,
sterol methyl transferase I enzyme activity,
sterol C4-demethylase enzyme activity,
obtusifoliol C14 α -demethylase enzyme activity,
sterol C5-desaturase enzyme activity, and

15 sterol methyl transferase II enzyme activity,
and a transcription termination signal sequence.

7. The recombinant vector of claim 6 wherein said vector is a plant expression vector.

8. A transformed host cell comprising a recombinant construct of claim 1.

9. The transformed host cell of claim 8 wherein said cell is a plant cell.

10. A transformed host cell comprising a recombinant vector of claim 6.

11. The transformed host cell according to claim 10 wherein said host cell is a plant cell.

12. A transformed host cell comprising a plant expression vector comprising:

(a) as operably linked components in the 5' to 3' direction, a promoter, a DNA sequence encoding a polypeptide having 3-hydroxy-3-methylglutaryl-Coenzyme A reductase enzyme activity, and a transcription termination signal sequence; and

(b) as operably linked components in the 5' to 3' direction, a promoter, a DNA sequence encoding at least one polypeptide having steroid pathway enzyme activity selected from the group consisting of

- squalene epoxidase enzyme activity,
- sterol methyl transferase I enzyme activity,
- sterol C4-demethylase enzyme activity,
- obtusifoliol C14 α -demethylase enzyme activity,
- sterol C5-desaturase enzyme activity, and
- sterol methyl transferase II enzyme activity, and

a transcription termination signal sequence.

13. The transformed host cell according to claim 12 wherein said host cell is a plant cell.

14. A cell culture comprising transformed host cells according to any of claims 8-13.

15. A plant comprising at least one transformed host cell of any of claims 8-13.

16. A plant according to claim 15 wherein said transformed host cell comprises a plant cell.

17. A storage organ, comprising at least one transformed host cell according to any one of claims 8-13.

18. A storage organ including at least one transformed host cell containing a recombinant vector comprising:

- (a) as operably linked components in the 5' to 3' direction, a promoter, a DNA sequence encoding at least one polypeptide having 3-hydroxy-3-methylglutaryl-Coenzyme A reductase enzyme activity, and a transcription termination signal sequence; and
- (b) as operably linked components in the 5' to 3' direction, a promoter, a DNA sequence encoding a polypeptide having steroid pathway enzyme activity selected from the group consisting of
 - squalene epoxidase enzyme activity,
 - sterol methyl transferase I enzyme activity,
 - sterol C4-demethylase enzyme activity,
 - obtusifoliol C14 α -demethylase enzyme activity,
 - sterol C5-desaturase enzyme activity, and
 - sterol methyl transferase II enzyme activity, anda transcription termination signal sequence.

19. The storage organ according to claim 18 wherein said recombinant vector is a plant expression vector.

20. A process of increasing the formation of steroid pathway products in a transformed host cell as compared to an otherwise identical non-transformed host cell comprising:

- 5 (1) transforming a host cell with a recombinant vector comprising
- (a) as operably linked components in the 5' to 3' direction, a promoter, a DNA sequence encoding a first polypeptide having
- 10 3-hydroxy-3-methylglutaryl-Coenzyme A reductase enzyme activity, and a transcription termination signal sequence; and
- (b) as operably linked components in the 5' to 3' direction, a promoter, a DNA
- 15 sequence encoding at least one polypeptide having steroid pathway enzyme activity selected from the group consisting of
- squalene epoxidase enzyme activity,
- 20 sterol methyl transferase I enzyme activity,
- sterol C4-demethylase enzyme activity,
- obtusifoliol C14 α -demethylase enzyme activity,
- 25 sterol C5-desaturase enzyme activity,
- and
- sterol methyl transferase II enzyme activity,
- and a transcription termination signal
- 30 sequence, and
- (2) regenerating said transformed plant cell into said transgenic plant.

090572.06001

21. The process according to claim 20 wherein said first encoded polypeptide comprises the catalytic region and at least a portion of the linker region but is free from the membrane binding region of an 3-
5 hydroxy-3-methylglutaryl-Coenzyme A reductase enzyme.

22. The process according to claim 20 wherein said promoters are promoters whose regulatory function is substantially unaffected by levels of squalene in said transgenic plant.

23. The process according to claim 20 wherein said plant cell is selected from the group consisting of canola, soybean, corn, tobacco, cotton, tomato, potato, safflower, sunflower, peanut, rape, flax, oil palm,
5 cuphea and alfalfa.

24. A transgenic plant produced in accordance with the process of claim 20.

25. A transgenic plant seed transformed with a vector comprising a DNA segment that encodes a polypeptide having 3-hydroxy-3-methylglutaryl-Coenzyme A reductase activity, and a DNA segment that encodes a polypeptide
5 having a steroid pathway enzyme activity selected from the group consisting of

squalene epoxidase enzyme activity,
sterol methyl transferase I enzyme activity,
sterol C4-demethylase enzyme activity,
10 obtusifoliol C14 α -demethylase enzyme activity,
sterol C5-desaturase enzyme activity, and
sterol methyl transferase II enzyme activity,

[illegible]

DNA encoding a polypeptide having 3-hydroxy-3-methylglutaryl-Coenzyme A reductase enzyme activity, wherein said plant contains an elevated level of total accumulated sterol, compared to an otherwise identical plant, the genome of which does not comprise said introduced DNA encoding a polypeptide encoding 3-hydroxy-3-methylglutaryl-Coenzyme A reductase enzyme activity; and

squalene epoxidase enzyme activity,
sterol methyl transferase I enzyme activity,
sterol C4-demethylase enzyme activity,
obtusifoliol C14 α -demethylase enzyme activity,
sterol C5-desaturase enzyme activity, and
sterol methyl transferase II enzyme activity,

20 wherein said introduced DNAs are operatively linked to
regulatory signals that cause seed-specific expression

[illegible]

28. A plant comprising introduced DNA encoding
(i) a polypeptide having 3-hydroxy-3-methylglutaryl-Coenzyme A reductase enzyme activity and
(ii) at least one additional polypeptide having
5 squalene epoxidase enzyme activity, sterol methyl transferase I enzyme activity, sterol C4-demethylase enzyme activity, obtusifoliol C14 α -demethylase enzyme

10 wherein said plant that produces a storage organ having
an elevated level of a sterol pathway product compared
to a corresponding transgenic or non-transgenic plant
that does not contain said introduced DNA.

5 corresponding transgenic plant that comprises
introduced DNA encoding a polypeptide having 3-hydroxy-
3-methylglutaryl-Coenzyme A reductase enzyme activity
but that does not contain introduced DNA encoding at
least one polypeptide having squalene epoxidase enzyme
10 activity, sterol methyl transferase I enzyme activity,
sterol C4-demethylase enzyme activity, obtusifolioside
C14 α -demethylase enzyme activity, sterol C5-desaturase
enzyme activity, sterol methyl transferase II enzyme
activity.

30. The plant of any one of claims 24 to 29, wherein said regulatory signals cause seed-specific expression of said introduced DNAs.

31. A seed of a plant according to any one of claims 24 to 30.

32. Progeny of a plant according to any one of claims 24 to 30.

34. A cell culture, comprising cells according to claim 33.

35. A plant produced from a seed according to claim
31.

36. A method of producing a plant comprising sexually crossing a plant according to any one of claims 15, 16 or 24-30 with a non-transformed plant.

37. A plant produced by the method of claim 36.

38. A plant according to any one of claims 24 to 30, wherein said plant is an apomictic plant.

39. A seed resulting from a cross of the plant of claim 38 with a nurse cultivar.

40. A uniform population of plants according to any one of claims 15, 16, 24-30, 37 or 38.

41. Seed of a plant produced by the method of claim
36.

42. A part, other than a seed, of a plant according to any one of claims 15, 16, 24-30, 37 or 38.

43. Oil containing at least one sterol pathway product, extracted from seed of a plant according to any one of claims 15, 16, 24-30, 37 or 38.

Variable	Mean	SD	Min	Max
Age	34.5	10.2	22	55
Gender	Male	Female	Male	Female
Marital Status	Married	Single	Married	Single
Education	High School	College	High School	College
Occupation	Manager	Worker	Manager	Worker
Income	\$20,000	\$30,000	\$10,000	\$40,000
Health Status	Good	Fair	Good	Fair
Stress Level	Low	High	Low	High
Life Satisfaction	High	Low	High	Low
Resilience	High	Low	High	Low
Optimism	High	Low	High	Low
Self-Efficacy	High	Low	High	Low
Emotional Stability	High	Low	High	Low
Problem Solving	High	Low	High	Low
Decision Making	High	Low	High	Low
Communication	High	Low	High	Low
Relationship Quality	High	Low	High	Low
Family Support	High	Low	High	Low
Community Support	High	Low	High	Low
Work-Life Balance	High	Low	High	Low
Job Satisfaction	High	Low	High	Low
Organizational Commitment	High	Low	High	Low
Employee Engagement	High	Low	High	Low
Productivity	High	Low	High	Low
Quality of Work Life	High	Low	High	Low
Overall Well-being	High	Low	High	Low

44. Oil according to claim 43, wherein sitosterol, at least one sitosterol ester, or mixtures thereof, comprise at least about 57% by weight of the total sterol compounds of said oil.

45. Oil according to claim 43, wherein sitosterol, at least one sitosterol ester, or mixtures thereof, comprise at least about 0.08% of the dry weight of said seed.

46. Oil according to claim 43, having a reduced amount of squalene, cycloartenol, 24-methylene cycloartenol, obtusifoliol, stigmasta-7-enol, campesterol, or mixtures thereof, compared to oil obtained from a
5 corresponding plant that does not contain introduced DNA encoding at least one polypeptide having squalene epoxidase enzyme activity, sterol methyl transferase I enzyme activity, a sterol C4-demethylase enzyme activity, obtusifoliol C14 α -demethylase enzyme
10 activity, sterol C5-desaturase enzyme activity, or sterol methyl transferase II enzyme activity; wherein the reduction is in the range of from about 10% to about 100% .

47. A sitosterol ester composition extracted from seed of a plant according to any one of claims 15, 16, 24-30, 37 or 38.

48. The sitosterol ester composition of claim 47, wherein an esterifying fatty acid has 2 to 22 carbon atoms in the main chain.

100290" 222880

5

5

5

5

55. An isolated DNA molecule, having a nucleotide sequence selected from the group consisting of:

56. An isolated DNA molecule, having a nucleotide sequence selected from the group consisting of:

- (a) disclosure obtusifoliol C14 α -demethylase SEQ ID NO:12, 14, or 17, or the complement thereof;
- 5 (b) a nucleotide sequence that hybridizes to said nucleotide sequence of (a) under a wash stringency equivalent to 0.5X SSC to 2X SSC, 0.1% SDS, at 55-65°C, and which encodes a polypeptide having obtusifoliol C14 α -demethylase enzymatic activity substantially
- 10 similar to that of the disclosed obtusifoliol C14 α -demethylase;
- (c) a nucleotide sequence encoding the same genetic information as said nucleotide sequence of (a), but which is degenerate in accordance with the degeneracy
- 15 of the genetic code; and

Table 1. Demographic characteristics of the study population	
Age (years)	65.0 ± 1.5
Gender (male/female)	10/10
Education (years)	12.0 ± 1.0
Occupation (white/blue)	10/10
Marital status (married/divorced/widowed)	10/10/0
Health status (good/fair/poor)	10/10/0
Smoking status (smoker/non-smoker)	10/10
Alcohol consumption (yes/no)	10/10
Family history of heart disease (yes/no)	10/10
Previous heart disease (yes/no)	10/10
Current heart disease (yes/no)	10/10
Current medication (yes/no)	10/10
Current exercise (yes/no)	10/10
Current diet (healthy/unhealthy)	10/10
Current stress (low/high)	10/10
Current sleep (good/poor)	10/10
Current mood (happy/sad)	10/10
Current energy (high/low)	10/10
Current weight (stable/gain/loss)	10/10
Current blood pressure (normal/high/low)	10/10
Current cholesterol (normal/high/low)	10/10
Current glucose (normal/high/low)	10/10
Current kidney function (normal/poor)	10/10
Current liver function (normal/poor)	10/10
Current lung function (normal/poor)	10/10
Current immune system (strong/weak)	10/10
Current skin health (good/poor)	10/10
Current hair health (good/poor)	10/10
Current teeth health (good/poor)	10/10
Current eyes health (good/poor)	10/10
Current ears health (good/poor)	10/10
Current nose health (good/poor)	10/10
Current throat health (good/poor)	10/10
Current stomach health (good/poor)	10/10
Current intestines health (good/poor)	10/10
Current bladder health (good/poor)	10/10
Current reproductive health (good/poor)	10/10
Current overall health (good/poor)	10/10

(d) a nucleotide sequence encoding the same genetic information as said nucleotide sequence of (b), but which is degenerate in accordance with the degeneracy of the genetic code.

57. A recombinant construct, comprising as operably linked components in the 5' to 3' direction, a seed-specific promoter, an isolated DNA molecule of claim 55 or 56, and a transcription termination signal sequence.

58. A recombinant vector comprising said recombinant construct of claim 57.

59. The recombinant vector of claim 58, which is a plant expression vector.

60. A transformed host cell, comprising said recombinant construct of claim 57.

61. The transformed host cell of claim 60, wherein said host cell is a plant cell.

62. A method of producing squalene epoxidase or obtusifoliol C14 α -demethylase, comprising culturing a transformed host cell of claim 60 for a time and under conditions conducive to the production of said squalene epoxidase or obtusifoliol C14 α -demethylase enzyme, and recovering said squalene epoxidase or obtusifoliol C14 α -demethylase produced thereby.

63. The transformed host cell of claim 8, further comprising a recombinant construct encoding a tocopherol synthesis pathway enzyme.

64. The transformed host cell of claim 10, further comprising a recombinant vector encoding a tocopherol synthesis pathway enzyme.

65. The transformed host cell of claim 64, wherein said tocopherol synthesis pathway enzyme is S-adenosylmethionine-dependent γ -tocopherol methyltransferase.

66. The transformed host cell of claim 64, wherein said vectors are plant expression vectors.

67. The storage organ of claim 18, wherein said at least one transformed host cell further contains a recombinant vector comprising as operably linked components, a promoter, a DNA sequence encoding a tocopherol synthesis pathway enzyme and a transcription termination sequence.

68. The storage organ of claim 18, wherein said tocopherol synthesis enzyme is S-adenosylmethionine-dependent γ -tocopherol methyltransferase.

69. A process of increasing the formation of steroid pathway products and tocopherols in a transformed host cell as compared to an otherwise identical non-transformed host cell comprising,

- 5 (A) transforming a host cell with:
- (1) a recombinant vector comprising
 - (a) as operably linked components in the 5' to 3' direction, a promoter, a DNA sequence encoding a first polypeptide having 3-hydroxy-3-methylglutaryl-Coenzyme
 - 10 A reductase enzyme activity, and a transcription termination signal sequence; and
 - (b) as operably linked components in the 5' to 3' direction, a promoter, a DNA sequence encoding at least one polypeptide having steroid pathway enzyme activity
 - 15 selected from the group consisting of squalene epoxidase enzyme activity, sterol methyl transferase I enzyme activity, sterol C4-demethylase enzyme activity, obtusifoliol C14 α -demethylase enzyme activity,
 - 20 sterol C5-desaturase enzyme activity, and sterol methyl transferase II enzyme activity, and a transcription termination signal sequence;
 - (2) a recombinant vector comprising as operably linked components, a promoter, a DNA sequence encoding at
 - 25 least one tocopherol synthesis pathway enzyme selected from the group consisting of 3-deoxy-D-arabino-heptulosonate-7-P synthase (DAHPSynthase), shikimate kinase,
 - 30 a prephenate dehydrogenase, 1-deoxy-d-xylulose 5-phosphate synthetase (DXS), 1-deoxy-d-xylulose 5-phosphate reductoisomerase (DXR), 4-diphosphocytidyl-2C-methyl-d-erythritol synthase (YgbP),
 - 35 4-diphosphocytidyl-2C-methyl-d-erythritol kinase (YchB),

T00290" 2/22/2004

the gene product of GcpE, LytB,

and a transcription termination sequence; and
55 (B) regenerating said transformed plant cell into said
transgenic plant.

70. The process of claim 69, wherein said tocopherol synthesis enzyme is S-adenosylmethionine-dependent γ -tocopherol methyltransferase.

71. A plant comprising at least one host cell of any
of claims 63-66.

72. A plant produced by the process of claim 69 or 70.

73. An oil from the plant of claim 71 or 72.

74. A seed from the plant of claim 71 or 72.

76. A plant produced by crossing the plant of any of claims 26-30, 35, 37, or 38 with a plant comprising a recombinant expression vector containing as operably linked components a promoter, a DNA sequence encoding a tocopherol synthesis pathway enzyme, and a transcription termination sequence.

77. The plant of claim 77, wherein said tocopherol synthesis enzyme is S-adenosylmethionine-dependent - tocopherol methyltransferase.